

Complete Listing of the Claims

1. (Currently amended) A method of characterizing single circulating epithelial cancer cells obtained from ~~a body fluid~~ about 5 mL to 75 mL of blood comprising:

~~the concurrent measurement of~~ concurrently measuring multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in said ~~single cells~~ using fluorescence microscopy.

2. (Currently amended) The method of claim 1, wherein said ~~single cells~~ is are isolated by density gradient centrifugation from a sample containing cells, said isolated cells are adhered onto a surface and fixed with a fixative solution, and said surface containing cells for characterization is incubated with said probes, wherein each probe reacts with a marker of the single cell, and any probe binding with a marker is examined by a microscope equipped with an optical filter set for identification of each specific marker.

Claims 3-8 (Canceled)

9. (Previously presented) The method of claim 2, wherein the surface for cell adherence is a microscope slide.

10. (Previously presented) The method of claim 2, wherein the fixative is selected from a group consisting of paraformaldehyde, formaldehyde, alcohol, or acetone.

11. (Original) The method of claim 1, wherein said probe is covalently linked to a fluorescent compound that emits a wavelength of light to create a fluorescent probe that binds to a cellular marker.

12. (Currently amended) The method of claim 11, wherein said fluorescent probe is selected from other probes with minimal overlapping emission spectra for concurrent use in characterizing said ~~single cells~~.

13. (Original) The method of claim 12, wherein said fluorescent probes are selected from a group consisting of a mixture of fluorescent probes that emit light of wavelengths between 400 nanometers and 850 nanometers, wherein said emission spectra can be distinguished from each other with the use of a microscope equipped with spectral filters that allow for elimination of most overlapping wavelengths of fluorescent light being emitted by each selected probe.

14. (Currently amended) The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 430 ~~nanometers~~ nanometers to 510 nanometers.

15. (Original) The method of claim 14, wherein said fluorescent probe emits light with a peak wavelength of about 470 nanometers.

16. (Currently amended) The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 482 ~~nanometers~~ nanometers to 562 nanometers.

Cl 17. (Original) The method of claim 16, wherein said fluorescent probe emits light with a peak wavelength of about 522 nanometers.

18. (Currently amended) The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 552 ~~nanometers~~ nanometers to 582 nanometers.

19. (Original) The method of claim 18, wherein said fluorescent probe emits light with a peak wavelength of about 567 nanometers.

20. (Currently amended) The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 577 ~~nanometers~~ nanometers to 657 nanometers.

21. (Original) The method of claim 20, wherein said fluorescent probe emits light with a peak wavelength of about 617 nanometers.

22. (Currently amended) The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 637 ~~nanometers~~ nanometers to 697 nanometers.

23. (Original) The method of claim 22, wherein said fluorescent probe emits light with a peak wavelength of about 667 nanometers.

24. (Currently amended) The method of claim 1, wherein said fluorescent probe emits light with wavelengths between 730 ~~nanometers~~ nanometers to 814 nanometers.

25. (Original) The method of claim 24, wherein said fluorescent probe emits light with a peak wavelength of about 772 nanometers.

26. (Canceled)

27. (Canceled)

28. (Original) The method of claim 13, wherein fluorescent compounds are selected from a group consisting of fluorescein isothiocyanate; CY3; CY3.5; CY5; CY5.5; AMCA; Tetramethylrhodamine Isothiocyanate; TEXAS RED™; R-Phycoerythrin; and Spectral Red.

29. (Original) The method of claim 1, wherein the probes comprise 4 fluorescent probes.

30. (Original) The method of claim 1, wherein the probes comprise 5 fluorescent probes.

31. (Original) The method of claim 1, wherein the probes comprise 6 fluorescent probes.

32. (Original) The method of claim 1, wherein the probes comprise 7 fluorescent probes.

33. (Original) The method of claim 1, wherein the probes comprise multiple fluorescent probes that emit light of different wavelengths with minimal interference between the wavelengths of emitted light when using appropriate filter set combinations that allow one marker to be distinguished from another when tested concurrently.

34. (Previously presented) The method of claim 1, wherein said probe is directed to a cellular target and is not a nucleic acid.

35. (Previously presented) The method of claim 34, wherein said probe comprises a protein or peptide.

36. (Original) The method of claim 35, wherein said probe is an antibody.

37. (Previously presented) The method of claim 1, wherein said probe is a nucleic acid directed to a cellular target.

38. (Previously presented) The method of claim 37, wherein said probe comprises DNA.

39. (Previously presented) The method of claim 37, wherein said probe comprises RNA.

40. (Previously presented) The method of claim 1, wherein said probes comprise
(i) probes which are directed to a cellular target and are not a nucleic acid,
(ii) probes which are a nucleic acid directed to a cellular target, or
(iii) a combination of (i) and (ii).

41. (Previously presented) The method of claim 40, wherein said probes are selected from the group consisting of identification probes, proliferation probes, cell cycle arrest probes, oncogenes, and hormonal probes.

42. (Canceled)

43. (Previously presented) The method of claim 40, wherein said probes comprises an epithelial cell-specific probe.

44. (Previously presented) The method of claim 40, wherein the probes comprise a tissue-specific probe.

45. (Original) The method of claim 1, wherein said cell is obtained from a mammal.

46. (Original) The method of claim 45, wherein said mammal is a human.

47. (Previously presented) The method of claim 40, wherein said probes are used to detect a hormone receptor or a hormone receptor gene for the enumeration of copy number.

48. (Original) The method of claim 47, wherein said hormone is an androgen.

49. (Original) The method of claim 47, wherein said hormone is an estrogen.

50. (Original) The method of claim 47, wherein said hormone is a progesterone.

51. (Original) The method of claim 1, wherein said cellular marker is an antigen.

52. (Original) The method of claim 51, wherein said cellular marker is a receptor.

53. (Currently amended) A method of characterizing a single circulating epithelial cancer cell preparation obtained from a body fluid about 5 to 75 ml of blood, said method comprising adhering a circulating epithelial cancer cell preparation to be characterized onto a surface, fixing said cell preparation with a fixative solution, incubating

said cell surface containing fixed cells with multiple probes directed to desired cellular markers, wherein said multiple probes have the ability to fluoresce when excited at different wavelengths, and examining the cells by fluorescence microscopy for identification of positive cells for each selected cellular marker by concurrent measurement of multiple cellular markers, wherein said cancer cell preparation is isolated from a body fluid using a negative selection process, wherein said circulating epithelial cancer cell is obtained .

54. (Currently amended) A method of establishing a characterization profile of a circulating epithelial cancer cell obtained from ~~a body fluid~~ about 5 to 75 ml of blood comprising characterizing a single cell environment by concurrent measurement of multiple cellular markers using fluorescent probes, wherein said probes emit different wavelengths of light to distinguish multiple cellular markers expressed in the single cell using fluorescence microscopy.

55. (Original) The method of claim 53, wherein said single cell is isolated by density gradient centrifugation from a sample containing cells, said isolated cells are adhered onto a surface and fixed with a fixative solution, and said surface containing cells for characterization is incubated with said probes, wherein each probe reacts with a marker of the single cell, and any probe binding with a marker is examined by a microscope equipped with an optical filter set for identification of each individual marker.

56. (Currently amended) The method of claim [[4]] 2, wherein cells are further isolated by a negative selection process.

57. (Canceled)

58. (Currently amended) The method of claim [[4]] 2, wherein cells are further isolated by a positive selection process, wherein a specific cell type is selected from a heterogeneous mixture of cells by an antibody that selectively binds to the specific cell type.

59. (Previously presented) The method of any one of claims 1, 53 and 54, wherein said circulating epithelial cancer cell is a prostatic cancer cell.


60. (Previously presented) The method of any one of claims 1, 53 and 54, wherein said circulating epithelial cancer cell is a breast cancer cell.

61. (Previously presented) The method of any one of claims 1, 53 and 54, wherein said circulating epithelial cancer cell is selected from the group consisting of liver, kidney, colon, rectum, gastric, esophageal, bladder, brain, ovary, pancreas and lung cancer cells.

62. (Canceled)

63. (Canceled)

64. (Previously presented) The method of any one of claims 1, 53 and 54, wherein said circulating epithelial cancer cell is obtained from about 5 to 25 ml of blood.

 65. (Previously presented) The method of any one of claims 1, 53 and 54, wherein said circulating epithelial cancer cell is obtained from about 15 to 25 ml of venous blood.

66. (Previously presented) The method of any one of claims 1, 53 and 54, wherein said circulating epithelial cancer cell is obtained from about 20 ml of blood.

67. (Canceled)

68. (Previously presented) The method of claim 64, wherein said circulating epithelial cancer cell is a prostatic cancer cell.

69. (Previously presented) The method of claim 65, wherein said circulating epithelial cancer cell is a prostatic cancer cell.

70. (Currently amended) The method of any one of claims 1, 53 and 54, wherein said probes are selected from the group consisting of:

- (a) tissue specific probes for determining the cellular origin of the cell;
- (b) probes specific cell tumor cell markers;
- (c) probes specific for aneuploidy;
- (d) probes specific for cellular markers of proliferation;
- (e) probes specific for cellular markers of cell growth inhibition;
- (f) probes specific for cell cycle arrest; and
- (g) probes specific for cellular markers of apoptosis; and
- (h) probes specific for hormonal receptors.

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